#### **ORIGINAL RESEARCH**



# Okra (Abelmoschus esculentus) Powder Production and Application in Gluten-Free Bread: Effect of Particle Size

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#### Abstract

Okra (*Abelmoschus esculentus*) has interesting nutritional and technological properties and is naturally gluten-free (GF). This study investigated the physicochemical properties of okra powder obtained by a low-temperature drying process and its impact on GF bread. Its potential synergy with other hydrocolloids (i.e., hydroxypropylmethylcellulose (HPMC) and Psyllium fibre (Psy)) was also studied. As the importance of powder particle size in food design is well known, whole okra powder (WOP;  $\leq 1000 \, \mu m$ ) and fine okra powder (FOP;  $\leq 250 \, \mu m$ ) were produced. Compared to the standard formulation, WOP and FOP doughs required less water to reach the desired dough consistency ( $200 \pm 20$  Brabender unit) and generally showed higher stability during mixing. Dough development was affected by HPMC more than okra powder particle size. Breads containing WOP or FOP in combination with HPMC exhibited high specific volume and soft texture, while the combination with Psy resulted in a less-developed, harder and darker bread. The combination with HPMC also guaranteed a longer shelf-life, regardless of okra powder particle size. These results may prove useful for the agri-food industry, as they demonstrate that okra can be used as an innovative natural hydrocolloid.

**Keywords** Low-temperature drying · Okra powder · Hydrocolloid · Particle size · Gluten-free · Bread quality

#### Introduction

Okra (Abelmoschus esculentus (L.) Moench) is a plant belonging to the Malvaceae family and is native to tropical and subtropical countries, but in recent years has spread to other areas, such as central and southern Italy. The ripe fruit looks like an elongated green pod (for this reason it is also known as "ladies' fingers") with violet shades, which can change with the variety of the plant. The whole pod is edible as well as its leaves, buds, and flowers (Farinde et al., 2006). According to Romdhane et al. (2020), okra pods consist of 81.9 g/100 g water, 8.2 g/100 g total dietary fibre (of which 4.7 g/100 g insoluble fibre and 3.4 g/100 g soluble fibre), 4.9 g/100 g carbohydrates, 3.6 g/100 g crude proteins, 1.5 g/100 g ash, 0.07 g/100 g fat and 50.6 kcal/100 g of energy. Furthermore, okra pods contain vitamins (e.g. vitamin A, B C, E and K), minerals (e.g. K and Ca), quercitin

Mucilage, mainly pectins (13.6 g/100 g dry based; dos Santos et al., 2019), can be extracted from fresh okra. Pectins are widely used in the food industry as an agricultural waste recovery agent (Archana et al., 2013), as well as a freezing and stabilizing agent in food emulsions (Alba et al., 2013) and as a flavouring and thickening agent in confectionery (Farinde et al., 2006). For instance, polysaccharides deriving from okra are used as emulsifiers, fat replacers and stabilisers in ice cream, chocolate, mousses, chips, cookies, cakes and tortillas (Datsomor et al., 2019; Kissiedu et al., 2020; Romanchik-Cerpovicz et al., 2002, 2006; Yuennan et al., 2014). However, since fresh okra is not always available in

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compounds, polyphenols, unsaturated fatty acids, essential amino acids, antioxidants, antimicrobials and few antinutritional compounds (Gemede et al., 2016); consequently, okra is sometimes used for cardiovascular, kidney and cerebral protection as well as antitumoural, anti-inflammatory, analgesic, antiulcerous, antibacterial, antidiabetic and anticholesterolemic purposes; okra is also efficient against asthenia and fatigue (Durazzo et al., 2019). Okra is also listed in the "Herbs for Voice Database" as one of the plants traditionally used for voice care, throat affections and respiratory impairments (Calcinoni et al., 2020).

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some countries and is highly perishable at room temperature because of its high moisture content, it is necessary to preserve the commodity. The traditional method for preserving okra involves slicing and sun drying (Falade & Omojola, 2010), then the fruit, with or without seeds, can be ground into powder. For example, Wirivutthikorn (2019, 2020), stabilised okra by a hot air oven treatment at 75 °C, while Sonkar et al. (2015) combined sun drying with hot air oven at 55–65 °C. At the industrial level, even other drying technologies (e.g. vacuum drying, microwave-vacuum, etc.) can be used to dehydrate perishable foods, as extensively described by Mujumdar and Law (2010). For instance, Alamri (2014) applied a freeze-drying process to obtain okra mucilage to add to a formulation for gluten-containing bread resulting in a dough with a greater capacity to absorb water and higher kneading tolerance but with weaker viscoelastic properties. Similarly, Bhat and Tharanathan (1987) used mucilage (extracted from okra pods and dried by solvent exchange) in wheat bread demonstrating that okra had interesting gelling and foaming properties due to its high water absorption and retention capacity. Morover, Xu et al. (2020) observed that wheat breads containing okra powder (obtained by air-drying at 40 °C) were characterised by reduced amylase activity, creating a greater sense of satiety because of the reduction in starch digestion rate.

In addition to its high nutritional value, widely discussed in the literature, okra is naturally gluten-free (GF) so it could be used (and valorised) for the production of GF products that have increased enormously in recent decades. In particular, GF breads are often characterised by structural and textural defects because of the lack of gluten, leading to dough that does not rise properly. Furthermore, GF breads are often characterised by crumbly texture that can impact negatively on consumer acceptance (Chakraborty et al., 2020). Hydrocolloids and other ingredients having a high affinity for water (e.g. hydroxypropylmethylcellulose (HPMC), guar gum, carboxymethylcellulose, xanthan, pectin, Psyllium (Psy), etc.) are generally used to modulate the viscoelastic properties of dough and the softness of the final product (Cappa et al., 2016a; Lazaridou et al., 2007; Mariotti et al., 2013; Sciarini et al., 2012). For instance, Mariotti et al. (2013) showed that HPMC limits both the diffusion and the loss of water from the bread crumb and the interactions between starch and protein macromolecules, resulting in a softer GF bread crumb and reducing staling kinetics during storage. Furthermore, the same authors revealed that the incorporation of ingredients having high dietary fibre content (such as buckwheat) improved dough development due to an increased viscosity and to the swelling, gelling and emulsion-forming properties of the flour used. Okra, thanks to its mucilage and fibre contents, could be a potential and unconventional ingredient to develop GF bread, eventually avoiding synthetic hydrocolloid (such as HPMC) that is not always well-accepted by consumers. However, as far as we know, there are no published studies concerning the use of okra in GF bread, especially as a powder.

The aim of this study was to produce okra powder by applying a low-temperature drying process to preserve heatsensitive materials (e.g. mucilage) before grinding. As powder particle size in food design is known to be important, whole okra powder (WOP; particle size  $\leq 1000~\mu m$ ) and fine okra powder (FOP; particle size  $\leq 250~\mu m$ ) were produced and their physicochemical properties were evaluated. The technological properties of the GF doughs containing the newly produced okra powders and the quality of the resultant fresh and stored breads were investigated in respect to a reference sample containing HPMC and Psy.

## **Materials and Methods**

#### **Materials**

Fresh okra (*Abelmoschus esculentus*) pods, belonging to the Clemson Spineless variety (origin: Honduras), were purchased from a local market in Milan (Italy). As pod size can affect proximate composition (Petropoulos et al., 2018), only pods having a length ranging from 107.7 to 174.3 mm were selected for the present study.

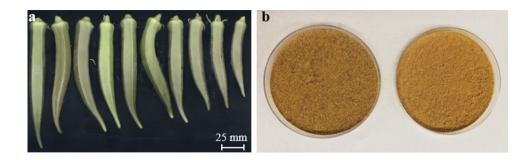
In order to produce GF breads, the following ingredients were used: rice flour (Beneo-Remy, Leuven-Wijgmaal, Belgium), corn starch (Roquette America Inc., IA, USA), pea proteins (IPP-F9 Cosucra, Warcoing, Belgium), Psy (Roeper GmbH, Germany), HPMC (UNIVAR S.p.A., Italy), extravirgin olive oil (Farchioni S.p.A., Giano dell'Umbria, Italy), granular sugar (Pellicano Comprabene S.p.A., Italy), sodium chloride (Carrefour Italia S.p.A, Italy), compressed yeast (Carrefour Italia S.p.A., Italy) and distilled water.

## **Okra Powder Production and Characterisation**

In order to obtain powders, fresh okra pods (Fig. 1) were washed and the ends (i.e. stem, calyx and apex) were removed; the remaining parts were transversely sliced (10 mm thick; FO), and dried at low-temperature ( $50\pm2$  °C) in a vacuum oven (WIPA, GEASS, Turin, Italy; operating at 0.987 Pa) down to approximately 15 g/100 g of moisture content. The particle size of okra powders were selected considering a compromise between the finest particles obtainable from okra pods by laboratory mills, as later reported, and the common particle size of traditional and unconventional flours (e.g. semolina, buckwheat, wheat flour, bean powder, etc.). For this purpose, the dried okra pieces were ground into powder at room temperature using a disc-mill (MLI 204, Buhler, Italy) and a blender (Heavy Duty Blender, Waring Commercial, USA) in order to obtain whole okra



**Fig. 1** Fresh okra pods (**a**) and powdered okra (**b**: whole okra powder, left; fine okra powder, right)



powder (WOP; Fig. 1) having a particle size smaller than 1000 µm. Since powder particle size is important in food design (Cappa et al., 2020; de la Hera et al., 2013a, b), WOP flour was refined excluding particles bigger than 250 µm, thus obtaining fine okra powder (FOP; Fig. 1). Sieving (50 g of powder per time) was conducted for 10 min at amplitude 9 using an analytical sieve shaker Octagon Digital (Endecotts Ltd., London, UK). The particle size distribution of okra powders was determined using a Mastersizer 3000® laser diffractometer (Malvern Instruments Limited, Worcestershire, UK) equipped with AERO S dry dispersion unit. Evaluations were performed at room temperature (25 °C), setting a constant refractive index of 1.52. Dispersion conditions were as follows: 100% air pressure at 2 bar, 2 mm hopper gap and 40% feed rate. According to Gnagne et al. (2017), particle size distributions were evaluated in terms of D10, D50 and D90, i.e. size (µm) below which 10, 50 or 90%, respectively, of the total volume of particles are found, and Span, describing the width of the distribution, calculated by the following formula: Span = ((D90 - D10)/D50). The okra powders were characterised in terms of moisture (Official Standard Method AACC 44-15A, 2000) and total nitrogen content (Kjeldahl method according to the AOAC, 2000). The protein contents were calculated adopting a conversion factor of 6.25.

In order to explore the technological properties of okra powders, water binding capacity (WBC), foaming capacity and stability were measured. WBC and foaming properties of HPMC and Psy were evaluated for comparison. WBC was determined by adding 50 mL of distilled water to a 0.5 g of sample in a tared 50 mL centrifuge tube. The tube was kept at room temperature and stirred intermittently every 10 min for up to 60 min; then the tube was centrifuged at  $2200 \times g$ for 10 min, and the excess water was carefully eliminated. WBC (g H<sub>2</sub>O/g sample as is) was calculated by subtracting the sample weight from the sample wet weight divided by the sample weight. Foaming capacity was measured by mixing 2.5 g of powdered sample with 50 mL of water then the suspension was stirred for 4 min, using a Whipped Cream Tester CT II mixer (Gerber Instruments, Switzerland), and the temperature was kept constant at 30 °C using a thermostatic bath. The height of the solution before and after mixing was also measured using a calliper. The suspension was then put into a graduated conical cylinder and the total volume, foam volume and liquid phase volume (mL) were measured immediately (t0) and after 30 and 60 min. Foaming capacity was expressed as overrun (%; difference between foam volume and initial solution volume with respect to the initial solution volume) and foaming stability (%; difference between initial foam volume and foam volume after 60 min with respect to the initial foam volume).

The colorimetric indices of powders (approximately 30 g) levelled in petri dishes were measured using a Minolta Chroma Meter II (Minolta, Osaka, Japan) equipped with standard Illuminant C. Results were expressed in the CIE L\*a\*b\* space: L\* (lightness; from black (0) to white (100)), a\* (from green (-) to red (+)) and b\* (from blue (-) to yellow (+)).

#### **Gluten-Free Dough and Bread Production**

Table 1 shows the recipe of GF breads and sample codes. The reference sample (STD) was formulated according to Cappa et al. (2016a) and contains of 1.5 g/100 g of HPMC and of Psy, respectively, as hydrocolloids. In the other breads, HPMC or Psy were substituted with FOP or WOP powders as specified in the sample code. The ingredient amount is expressed on a total recipe weight basis, water excluded.

## **Dough Mixing and Leavening Properties**

The mixing properties of doughs were investigated with a Brabender Farinograph (Brabender® OHG, Duisburg, Germany) set at 30 °C. The dry ingredients (273 g) were pre-mixed for 5 min, before adding the yeast (previously suspended in a part of water), oil and water up to the desired dough consistency of  $200 \pm 20$  Brabender® unit (BU) in accordance with Cappa et al. (2013, 2016a). Kneading was performed for 15 min. For each formulation, water absorption (%; intended as the amount of water required to reach the desired consistency), initial and final consistencies (evaluated at 5 and 15 min, respectively) and kneading stability (%; calculated as the difference between the initial and the



**Table 1** GF bread recipe and sample code

Ingredients (g/100 g)	STD	HPMC+WOP	HPMC+FOP	Psy + WOP	Psy + FOP
Rice flour	38	38	38	38	38
Corn starch	38	38	38	38	38
Pea proteins	6	6	6	6	6
HPMC	1.5	1.5	1.5	0	0
Psy	1.5	0	0	1.5	1.5
WOP	0	1.5	0	1.5	0
FOP	0	0	1.5	0	1.5
Sugar	4	4	4	4	4
Sodium chloride	2	2	2	2	2
Olive oil	6	6	6	6	6
Compressed yeast	3	3	3	3	3

HPMC hydroxypropylmethylcellulose, Psy Psyllium fibre, WOP whole okra powder, FOP fine okra powder

final consistencies with respect to the initial consistency) were determined. To evaluate the leavening behaviour, 300 g of the dough produced with the Brabender Farinograph was placed into the Rheofermentometer F3 Chopin (Chopin SA, Villeneuve-La-Garenne, France) and left to rise for 1 h at 35 °C. Dough development (mm) and gas production (mm  $\rm H_2O$ ) were continuously measured during leavening, while the volume of gas produced and retained (mL) were determined at the end of the process. The time when the dough starts to lose the  $\rm CO_2$  produced by yeast was measured to determine the proper dough leavening time for the baking trials.

#### **Breadmaking Procedure**

Dough produced in the Brabender Farinograph was collected, divided into portions of 60 g and placed into oiled metal moulds ( $100\times60\times45$  mm). According to the rheofermentographic data, the dough was leavened at 35 °C for 40 min (Whirlpool, EMEA S.p.A., Biandronno, Varese, Italy) and baked in an electric static oven (Whirlpool, EMEA S.p.A., Biandronno, Varese, Italy) for 30 min at 175 °C. At the end of baking, the loaves were cooled for 30 min at room temperature, before being removed from the moulds. The quality of the GF breads was evaluated immediately after cooling (t0, fresh bread) and after 24 h (t24) and 48 h (t48) of storage at 25 °C in unsealed paper bags to simulate home storage.

## **GF Bread Characterisation**

Breads were characterised as follows: height (mm; using a calliper, taking measurements at the maximum of the loaf); specific volume (mL/g; AACC Official Standard Method 10–05.01, 2000, replacing rapeseeds with sesame seeds); slice and crumb moisture (g/100 g; AACC Official Standard Method 44-15A, 2000); crumb water activity (AquaLab

Series CX-3, Decagon Devices Inc. Pullman, USA); baking loss (%; computed as the difference between the weight of the dough before leavening and the weight of the fresh bread with respect to the dough weight); storage loss (%; computed as the difference between fresh and stored bread with respect to the fresh weight). Crust and crumb colour were measured using a tristimulus colour light analyser (Minolta Chroma Meter II Reflectance; Minolta, Osaka, Japan) as previously described for okra powder characterisation. For each analysis, at least six replicates (n=6) were performed for each bread recipe. Crumb porosity was investigated using Image Analysis: a central crumb portion (crop size = 442.7 mm<sup>2</sup>) was selected from each bread slice previously scanned in 256 grey scale levels and 600 dpi resolution using an Epson Perfection V850pro scanner (Seiko Epson Corporation, Suwa, Japan). Images were processed using a specific software (Image Pro-Plus 7.0; Media Cybernetics Inc., Rockville, MD, USA) and the objects (holes) were identified, counted and classified into five classes on the basis of their size: (1)  $0.1 \le x < 0.2 \text{ mm}^2$ , (2)  $0.2 \le x < 0.5 \text{ mm}^2$ , (3)  $0.5 \le x < 1 \text{ mm}^2$ , (4)  $1 \le x < 5 \text{ mm}^2$  and (5)  $5 \le x < 10 \text{ mm}^2$ . The following parameters were measured: hole number for each class (%; expressed as the percentage of holes having a selected size with respect to the total number of crop holes) and crumb porosity (%; expressed as the percentage of the total alveolate area of the crop with respect to the total surface crop). Bread crumb softness was investigated through a compression test using a TA-HDplus Texture Analyser (Stable Micro Systems, Surrey, UK), equipped with a 500 N load cell. The Texture Exponent TEE32 V 3.0.4.0 Software (Stable Micro System, UK) was used to control the instrument and to process data. Breads were sliced (20 mm thick) and each slice was compressed up to 10% deformation at a compression speed of 1 mm/s, using a spherical probe. The trigger force was equal to 10 g. Crumb resistance (N) to a deformation of 10% was measured as indication of crumb hardness. At least six replicates (n=6) were performed for



each bread recipe. During storage, GF bread loaves were also characterised in terms of slice and crumb moisture, crumb water activity and softness, as previously reported.

# **Statistical Analysis**

If not stated otherwise, triplicate measurements were performed for each sample. Results were expressed as mean  $\pm$  standard deviation (SD) values. All data were subjected to one-way analysis of variance (ANOVA), followed by the least significant difference (LSD) test to identify significant differences among the samples (P<0.05). Correlation analysis among variables was performed following the Pearson approach. The Pearson product-moment of correlation (r) was calculated, together with the P value of statistical significance. Data were processed by STATGRAPHIC® Centurion 18 (Statpoint Technologies Inc., The Plains, VA, USA).

#### **Results and Discussion**

#### **Fresh and Powdered Okra Characteristics**

Table 2 reports the physicochemical properties of fresh and powdered okra. In agreement with Romdhane et al. (2020), dos Santos et al. (2019) and Falade and Omojola (2010) who reported moisture values of 81.9, 84.0 and 90.2 g/100 g, respectively, FO had a moisture content of 90.6 g/100 g that inevitably causes a fast deterioration of the pod at room temperature. As regards protein content, FO had a value of  $16.26 \pm 0.03$  g/100 g db (dry basis), and this is in agreement with Petropoulos et al. (2018) who stated that okra pods were very rich in protein compared to other fresh vegetables suggesting that okra pods could serve as a good source of

protein in human diet. However, a wide range of protein content has been reported in the literature for fresh okra: 10.25-26.16 g/100 g db, 19.61 and 29.74 g/100 g db (dos Santos et al., 2019; Gemede et al., 2016; Romdhane et al., 2020, respectively). For instance, Petropoulos et al. (2018) showed that protein content varied with pod size: small pods (30-50 mm) had 17.25-17.92 g/100 g db while large ones (> 70 mm) had 14.62–15.75 g/100 g db. As expected, powdered samples (WOP and FOP; Table 2) had a moisture content around 15 g/100 g, confirming that the lowtemperature drying process applied resulted in more stable products at room temperature. Furthermore, their protein contents resulted similar to the values found for thermally treated okra powders (18.98 to 25.65 g/100 g db; dos Santos et al., 2019), regardless of the cultivar of the plant and ripening stage. As okra powders have been suggested as new ingredients for baking application, their particle size and technological properties were evaluated. According to the milling and sieving conditions applied, WOP had 90% of the total particles smaller than 806 µm, while FOP was characterised by a finest particle size ( $D_{90} = 310 \mu m$ ). Even if WOP had smaller surface areas it showed significantly higher WBC than FOP (Table 2); this can be explained by the fact that most of the tissues found in living plants are irregular in terms of composition and resistance to fracture (Xu et al., 2020); thus, after drying and milling, the different fractions collected might differ due to the varying proportions of the tissue parts. WBC of okra powders were lower than Psy  $(36.7 \pm 0.8 \text{ g H}_2\text{O/g})$ , while their foaming properties were comparable (overrun of 0.8% for WOP, FOP and Psy; data not shown); on the contrary, HPMC was able to bind all the water added during the WBC assay and showed an overrun of  $34.7 \pm 2.3\%$  with a foam characterised by high stability (100% after 60 min; data not shown).

**Table 2** Physicochemical properties of fresh okra (FO) and newly produced powders (whole okra powder, WOP and fine okra powder, FOP)

	FO	WOP	FOP
Moisture (g/100 g)	$90.60 \pm 0.40c$	15.62±0.03a	$16.41 \pm 0.03b$
Protein content (g/100 g db)	$16.26 \pm 0.03a$	$22.00 \pm 0.58$ b	$22.74 \pm 0.88b$
WBC (g $H_2O/g$ )	-	$21.32 \pm 1.37$ b	$15.10 \pm 0.33a$
Overrun (%)	-	$0.80 \pm 0.10$ a	$0.80 \pm 0.10 a$
L*	$47.33 \pm 4.32a$	$63.48 \pm 0.65c$	$58.29 \pm 2.15$ b
a*	$-14.93 \pm 1.74a$	$-4.52 \pm 0.15$ b	$-2.73 \pm 0.31c$
b*	$18.72 \pm 3.52a$	$19.50 \pm 0.43a$	$20.59 \pm 1.45a$
D10 (µm)	-	$38.3 \pm 4.9b$	$17.3 \pm 0.8a$
D50 (µm)	-	$379.2 \pm 19.6b$	$118.0 \pm 6.6a$
D90 (μm)	-	$837.2 \pm 15.9$ b	$310.3 \pm 2.0a$
Span	-	$2.1 \pm 0.1a$	$2.5 \pm 0.1b$

Mean values in the same row with different letters (a–c) are significantly different (P < 0.05)

db dry basis, WBC water binding capacity, L\*, lightness; a\*, green-red index; b\* blue-yellow index,  $D_{10}$ ,  $D_{50}$  and  $D_{90}$ , size below which 10, 50 or 90%, respectively, of the total volume of particles are found; Span, width of the particle size distribution curve; -, measured only for powders



Table 3 Dough kneading properties

	STD	HPMC+WOP	HPMC+FOP	Psy+WOP	Psy+FOP
Water absorption (%)	$79.25 \pm 0.35c$	$64.60 \pm 0.99a$	$64.40 \pm 1.70a$	$67.67 \pm 1.27$ b	67.15 ± 1.63ab
Initial consistency (BU)	$208.00 \pm 2.83a$	$201.00 \pm 1.41a$	$195.00 \pm 5.66a$	$194.00 \pm 9.90a$	$201.50 \pm 13.44a$
Final consistency (BU)	$200.00 \pm 2.83a$	$197.00 \pm 2.83a$	$193.00 \pm 5.66a$	$188.50 \pm 7.78a$	$188.00 \pm 7.07a$
Kneading stability (%)	$96.16 \pm 0.05$ ab	$98.02 \pm 2.10b$	$98.97 \pm 0.03b$	$97.19 \pm 0.95$ ab	$93.39 \pm 2.72a$

See Table 1 for sample recipe and code. Mean values in the same row with different letters (a–c) are significantly different (P<0.05) BU Brabender unit, HPMC hydroxypropylmethylcellulose, WOP whole okra powder, FOP fine okra powder, Psy Psyllium fibre

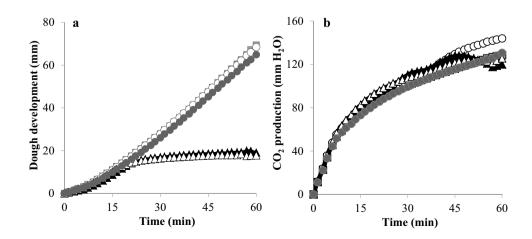
As one of the first quality indicator for consumer is the product colour and it can be altered by thermal processing (e.g., Maillard reaction), the colour of the newly produced okra powders versus FO was evaluated (Table 2). Compared to FO, the process applied (i.e., drying and grinding) significantly (P < 0.05) affected sample colour leading to an increase in brightness (L\*) associated with a decrease of the green component  $(-a^*)$  with respect to the red  $(+a^*)$ ; no significant differences were found for the yellow index (b\*). Comparing two okra powders having different particle sizes, WOP scored significantly (P < 0.05) brighter (63.48) vs. 58.29, respectively) and was characterised by a higher value for green (-4.52 vs. -2.73, respectively) than FOP, whereas no differences were found for yellowness. These results are in agreement with dos Santos et al. (2019) who characterised okra powder lyophilised and dried in a convective oven at different processing temperatures resulting in L\* values in a range of 58.41–70.04, a\* values between -5.09 and -1.25, and b\* values from 20.73 to 24.51.

## **Dough Quality Evaluation**

The technological properties of dough are reported in Table 3 and Fig. 2. The amount of water required to reach the desired consistency  $(200\pm20~\mathrm{UB}$  according to Cappa et al., 2013, 2016a) ranged from 64.40 to 79.25% according to the ingredients used having different WBCs. Indeed, the

standard formulation containing HPMC and Psy required the greatest quantity of water, while samples containing okra powders bound less water. Furthermore, in accordance with WBC values, dough containing WOP required slightly more water than FOP-dough; this result is in agreement also with de la Hera et al. (2014) who studied the effect of rice flour particle size on gluten-free bread. Despite being gluten-free, all the samples exhibited high dough stability (>93%) during kneading that increased (up to 98%) by substituting Psy with WOP or FOP. The ability of okra to increase dough stability was also observed by Bhat and Tharanathan (1987) and Alamri (2014) in wheat dough. All the samples showed also high CO<sub>2</sub> production (Fig. 2; reaching volumes of 862-935 mL of CO<sub>2</sub>, data not shown), suggesting that yeast was fermenting sugars and the proofing conditions were suitable for their growth. No correlation was found between dough development and gas amount indicating that the leavening performance of the yeast is not the only parameter to consider, indeed the capability of the dough to retain gas is also critical. In fact, STD, HPMC+FOP and HPMC+WOP samples, which had very high retention coefficients (>99%, data not shown), increased progressively during leavening (with final dough heights of 69.4, 68.4 and 64.9 mm, respectively; Fig. 2); on the contrary, samples without-HPMC resulted in lower dough developments (19.1 and 17.8 mm for Psy + WOP and Psy + FOP, respectively) suggesting that the rheological properties (e.g. viscoelasticity)

Fig. 2 Leavening properties (dough development, **a**; CO<sub>2</sub> production, **b**) of the dough as a function of leavening time. Samples: STD, grey square; HPMC+WOP, grey round dot; HPMC+FOP, grey round empty dot; Psy+WOP, black triangle; Psy+FOP, black empty triangle





of dough containing HPMC are more suitable to produce a well-developed bread. Furthermore, as Fig. 2 indicates, all the doughs started rising immediately; this is typical of dough containing compressed yeast, while sourdough generally required a longer time to ferment sugars (Cappa et al., 2016b). Additionally, at the beginning of the leavening all the samples proceeded similarly, then, after approximately 25 min of leavening, the dough height of samples without HPMC plateaued. This behaviour is not surprising as HPMC is one of the most popular hydrocolloids used in glutenfree bread production (Cappa et al., 2013; Hager & Arendt, 2013; Mancebo et al., 2015; Mariotti et al., 2013) and could be explained by the fact that Psy and okra powders possess mainly thickening properties rather than the unique foaming properties of HPMC, as previously discussed. Lastly, all the samples exhibited high CO<sub>2</sub> retention (>98%, data not shown) with no leaching of gas during the test or only tiny losses after 40 min, thus the dough leavening time for the subsequent baking trials was set for 40 min.

# **Bread Quality Evaluation**

Figure 3 shows images of the central slice of fresh bread samples, while bread characterisation is summarised in Table 4. As expected, for all the samples, the crust was darker and characterised by higher red and yellow values in comparison to the crumb; this is mainly due to the Maillard reaction which is more accentuated in the crust which reached higher temperatures, as was also reported by Giovanelli and Cappa (2021). Furthermore, the bread formulation used (i.e. HPMC, Psy, WOP or FOP addition) affected crust and crumb colour. Indeed, breads containing Psy and okra powders resulted significantly (P < 0.05) darker due to the original colour of both ingredients tending to green/brown and characterised by lower L\* values (data not shown) compared to the samples containing HPMC which was bright white. These data were in agreement with Alamri (2014) and Xu et al. (2020) who also demonstrated that bread with okra gum or powder was darker. Even if WOP and FOP showed significantly (P < 0.05) different chromatic coordinates (Table 2), slight differences of the colour of bread containing okra powders were observed according to their particle size, probably due to the low amount of okra powder added in the bread recipe (Table 1).

Figure 3 shows all breads with a well-defined crust and crumb without particular defects (e.g. cracking or crumb discontinuities), and no significant differences were evidenced for crumb porosity with the exception of STD which had the highest value (29 vs. 22–24%; Table 4); furthermore, it was observed that adding HPMC results in bread with the finest crumb structure (> 33% of the holes had the smallest size), whereas samples containing FOP and WOP in combination with Psy showed a crumb structure defined by bigger holes (37 and 48% larger than 0.5 mm² for Psy + WOP and Psy + FOP, respectively); larger crumb holes were also found by Xu et al. (2020) in wheat bread containing okra powder.

As regards crumb moisture, according to the farinographic water absorption values, STD had the highest moisture content followed by samples containing Psy + okra, and HPMC + okra; indeed a positive linear correlation (r = 0.928, P < 0.05) was found between dough water absorption and bread crumb moisture, while no correlation was found for bread weight and specific volume. No significant differences in bread weights were found for samples containing HPMC, while those containing Psy and okra powders were heavier; this could be due to the presence of fibre which bound water without evaporating excessively during the cooking phase; this behaviour was confirmed by the lowest baking loss and the bread moisture content of Psy + WOP and Psy + FOP which were the highest even if they showed intermediate farinographic water absorption values (Table 3). However, the presence of pericarp parts of Psy seed and okra pod weakened the non-gluten network of the dough resulting in less developed bread (Table 3); this is in agreement with Hřivna et al. (2018) who demonstrated that the presence of bran in wheat dough led to a lower development and lower specific volume of the final bread. The combination of HPMC and okra powders resulted in well-developed breads characterised by high values for height and specific volume (Table 4 and Fig. 3); furthermore, as expected, okra powder particle size affected the bread structure since the powder with the smaller particle size (i.e. FOP) resulted in

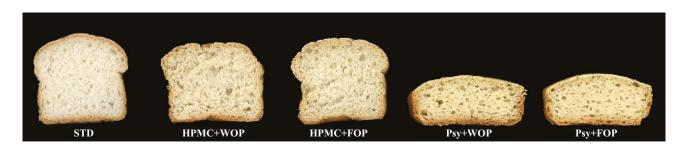


Fig. 3 Images of the central slice of fresh GF bread. See Table 1 for sample recipe and code

Table 4 Quality evaluation of fresh GF bread

	STD	HPMC+WOP	HPMC+FOP	Psy+WOP	Psy+FOP
Crust L*	$72.25 \pm 3.38c$	$72.09 \pm 4.59c$	$68.80 \pm 2.91b$	$60.08 \pm 1.10a$	61.43 ± 1.28a
Crust a*	$2.89 \pm 1.07b$	$2.17 \pm 1.45$ ab	$2.79 \pm 0.86$ b	$2.88 \pm 0.86$ b	$1.75 \pm 0.56a$
Crust b*	$24.61 \pm 2.24a$	$24.14 \pm 2.54a$	$25.23 \pm 2.15a$	$30.34 \pm 0.88b$	$29.03 \pm 1.37$ b
Crumb L*	$84.57 \pm 2.50d$	$77.59 \pm 2.27c$	$76.47 \pm 1.90c$	$64.97 \pm 1.33$ b	$62.20 \pm 2.83a$
Crumb a*	$-2.90 \pm 0.55a$	$-2.91 \pm 0.43a$	$-3.01 \pm 0.23a$	$-2.48 \pm 0.20$ b	$-2.17 \pm 0.40$ b
Crumb b*	$9.08 \pm 0.87a$	$13.96 \pm 1.38$ bc	$13.23 \pm 1.06$ b	$15.38 \pm 0.46d$	$14.81 \pm 0.60$ cd
Baking loss (%)	$22.29 \pm 1.34c$	$21.57 \pm 0.93b$	$22.38 \pm 1.53$ bc	$18.87 \pm 1.39a$	$19.82 \pm 0.88a$
Bread weight (g)	$46.68 \pm 1.11a$	$47.40 \pm 0.72a$	$46.99 \pm 0.86a$	$48.54 \pm 0.51$ b	$48.60 \pm 0.47$ b
Crumb water activity	$0.985 \pm 0.005$ b	$0.980 \pm 0.003$ ab	$0.973 \pm 0.005a$	$0.977 \pm 0.011$ ab	$0.975 \pm 0.011$ ab
Crumb moisture (g/100 g)	$49.52 \pm 0.67c$	$45.47 \pm 1.27a$	$45.96 \pm 0.42a$	$47.15 \pm 0.83$ b	$47.30 \pm 0.15$ b
Slice moisture (g/100 g)	$36.31 \pm 0.30e$	$32.64 \pm 1.65$ b	$32.52 \pm 0.87a$	$34.93 \pm 1.83c$	$36.23 \pm 0.55$ d
Bread height (mm)	$53.88 \pm 3.13d$	$45.90 \pm 1.79$ b	$50.39 \pm 2.70c$	$24.90 \pm 0.59a$	$25.39 \pm 0.70a$
Bread specific volume (mL/g)	$3.99 \pm 0.24d$	$3.07 \pm 0.28c$	$3.89 \pm 0.32d$	$1.88 \pm 0.31a$	$2.32 \pm 0.30$ b
Crumb porosity (%)	$29.06 \pm 0.08b$	$23.52 \pm 1.74a$	$21.76 \pm 0.21a$	$23.16 \pm 0.23a$	$22.91 \pm 1.56a$
Holes class 1 (%)	$35.1 \pm 3.4e$	$33.4 \pm 4.0d$	$32.7 \pm 3.8c$	$27.4 \pm 1.7b$	$22.3 \pm 4.2a$
Holes class 2 (%)	$28.8 \pm 3.7b$	$28.7 \pm 1.5a$	$30.3 \pm 4.1d$	$35.4 \pm 2.6e$	$29.6 \pm 5.8c$
Holes class 3 (%)	$16.7 \pm 3.0$ b	$17.4 \pm 1.2d$	$16.6 \pm 3.7a$	$16.9 \pm 0.2c$	$22.4 \pm 1.6e$
Holes class 4 (%)	$16.4 \pm 2.3a$	$18.8 \pm 3.3b$	$18.9 \pm 5.8c$	$19.2 \pm 2.9 d$	$24.9 \pm 5.9e$
Holes class 5 (%)	$2.7 \pm 1.4d$	$1.4 \pm 0.8$ c	$1.1 \pm 0.7b$	$1.1 \pm 0.8b$	$0.9 \pm 0.1a$
Hardness (N)	$0.256 \pm 0.042a$	$0.315 \pm 0.032a$	$0.284 \pm 0.027a$	$3.214 \pm 1.332c$	$1.975 \pm 0.496$ b

See Table 1 for sample formulation and code. L\*, lightness; a\*, green-red index; b\*, blue-yellow index; class 1:  $0.1 \le x < 0.2$  mm<sup>2</sup>; class 2:  $0.2 \le x < 0.5$  mm<sup>2</sup>; class 3:  $0.5 \le x < 1$  mm<sup>2</sup>; class 4:  $1 \le x < 5$  mm<sup>2</sup>; and class 5:  $5 \le x < 10$  mm<sup>2</sup>. Mean values in the same row with different letters (a-e) are significantly different (P < 0.05)

HPMC hydroxypropylmethylcellulose, WOP whole okra powder, FOP fine okra powder, Psy Psyllium fibre

significantly (P < 0.05) higher specific volume in comparison to WOP (3.89 and 3.07 mL/g, respectively) and was not significantly different from STD bread. Different results were found by de la Hera et al. (2013a) who studied the influence of rice flour particle size on the quality of gluten-free rice bread founding that the finer the powder, the poorer its bread-making properties. Such a discrepancy can be due to the different composition (e.g. protein, starch and fibre content) of okra powder and rice flour and their particle size; in fact, rice flours were very fine (< 80, 80–180 and > 180 µm) in comparison with okra powders. Moreover, Xu et al. (2020) evidenced that very fine (i.e.  $< 150 \mu m$ ) particle sizes were not suitable for bread making, since they somehow inhibited the formation of a proper network resulting in a decreased bread volume and increased hardness, while the okra powder fractions obtained from 150 and 270 µm mesh screens—thus similar to FOP—performed better. Nevertheless, according to the bread recipe, a huge range of specific volume values (1.88-3.99 mL/g) were obtained for the experimental GF breads; these values were similar to or even higher than published data related to gluten-free bread (Belorio & Gómez, 2020; Cappa et al., 2016a; Hager & Arendt, 2013; Mariotti et al., 2013; Sciarini et al., 2012) indicating that formulation and process conditions used are suitable to produce well-developed bread. A negative linear correlation was found between specific volume and bread hardness (r=0.925, P<0.05) suggesting that volume, one of the visual attributes consumers use to measure bread quality, can be a good indicator of bread softness for pan bread. The evaluation of the crumb texture further indicated that okra powder can be successfully used in combination with HPMC to develop soft GF bread (having hardness not significantly different from STD) and powder particle size somehow affected the texture: lower values for hardness characterised samples containing FOP compared to WOP. Similarly Xu et al. (2020) found that high quality wheat breads could be obtained by using flours having 150–270  $\mu$ m particle size, especially from okra seed fractions.

#### **Bread Quality Evaluation During Storage**

In order to evaluate the effects of okra powder addition on bread shelf-life, samples were also characterised after 24 h (t24) and 48 h (t48) of storage at 25 °C in unsealed paper bags simulating home storage. As expected, all breads dehydrated during storage (Table 5): high bread weight loss (6–19%), low water activity (0.866–0.975) and low crumb and slice moisture (25–46% and 17–33%, respectively) were



Table 5 Quality evaluation of GF bread during storage

	Storage time (h)	STD	HPMC + WOP	HPMC+FOP	Psy+WOP	Psy+FOP
Weight loss (%)	24	9.49 ± 1.36a	8.39 ± 1.29a	$9.84 \pm 0.52a$	7.12±4.21a	$6.35 \pm 3.10a$
	48	$18.81 \pm 0.01d$	$17.28 \pm 0.03c$	$18.15 \pm 0.11$ cd	$11.95 \pm 0.82a$	$14.15 \pm 0.06$ b
Water activity	24	$0.975 \pm 0.002d$	$0.968 \pm 0.002c$	$0.955 \pm 0.001a$	$0.961 \pm 0.001b$	$0.953 \pm 0.003$ a
	48	$0.933 \pm 0.004c$	$0.877 \pm 0.013$ b	$0.866 \pm 0.002a$	$0.940 \pm 0.004c$	$0.943 \pm 0.003$ c
Crumb moisture (g/100 g)	24	$46.00 \pm 2.62$ b	$40.73 \pm 0.26a$	$38.76 \pm 2.17a$	$42.26 \pm 0.46$ ab	$42.32 \pm 0.77$ ab
	48	$33.94 \pm 0.06b$	$25.64 \pm 0.50a$	$25.52 \pm 0.51a$	$36.93 \pm 1.53c$	$35.71 \pm 0.30$ bc
Slice moisture (g/100 g)	24	$33.50 \pm 0.43d$	$29.03 \pm 0.67$ b	$23.75 \pm 0.76a$	$32.70 \pm 0.87$ cd	$30.98 \pm 1.57$ bc
	48	$24.72 \pm 0.14$ bc	$21.78 \pm 1.17b$	$17.45 \pm 1.07a$	$27.78 \pm 2.06c$	$25.30 \pm 1.95$ bc
Hardness (N)	24	$0.495 \pm 0.086a$	$0.665 \pm 0.083a$	$0.923 \pm 0.097a$	$13.655 \pm 1.739$ b	$16.336 \pm 2.174c$
	48	$0.881 \pm 0.039a$	$1.521 \pm 0.366a$	$2.060 \pm 0.357a$	$26.672 \pm 3.592$ b	$28.044 \pm 3.506$ b

See Table 1 for sample formulation and code. Mean values in the same row with different letters (a–d) are significantly different (P<0.05) HPMC hydroxypropylmethylcellulose, WOP whole okra powder, FOP fine okra powder, Psy Psyllium fibre

found; consequently, a hardening of the crumb was evidenced. Samples containing WOP and FOP in combination with Psy exhibited faster staling kinetics reaching values for hardness up to 33 times higher than STD, while the combination of okra powder with HPMC resulted in soft bread that was not significantly different from the reference samples for both storage times. Contrary to expectation, the okra powder having the largest particle size was able to constrain bread hardening better than fine powder, probably due to its higher water binding capacity.

## **Conclusion**

As far as we know, this study is the first to investigate the effect of okra powder of varying particle size on the technological quality of GF dough and bread. Results indicated that okra powder can be used in combination with HPMC to produce GF bread characterised by high specific volume and soft texture typical of pan bread. On the contrary, the combination of okra powder with Psy produced a more compact bread (i.e. low specific volume and high crumb hardness). Powder particle size affected dough and bread properties: samples containing the finest okra powder required less water to reach 200 BU and were characterised by higher dough development and bread specific volume, crumb moisture and crumb softness; however, the largest particle size powder was slightly more effective in limiting bread hardening during storage. Furthermore, the formulations containing okra powders and Psy, while aiming to replace HPMC (synthetic hydrocolloid) with natural ingredients, also yielded interesting results from a technological point of view (i.e. colour and crumb porosity) even if the crumb was more compact (i.e. higher hardness). Further studies should be conducted in order to optimise the amount of okra powder (and water) to use and to explore combinations with other non-synthetic hydrocolloids in order to obtain green labels. In conclusion, the application of okra powder in other bakery and confectionery products seems worthy of investigation since not much data is currently available in the literature, especially regarding GF products.

**Author Contribution** Deborah Tufaro: investigation, methodology, formal analysis, writing (original draft). Angela Bassoli: resources, supervision. Carola Cappa: conceptualisation, methodology, formal analysis, data curation, resources, project administration, writing (original draft).

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**Data Availability** The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

#### **Declarations**

**Conflict of Interest** The authors declare no competing interests.

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